CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF CHITOSAN COMPOSITE BIODEGRADABLE FILMS WITH ADDITION OF CLOVE ESSENTIAL OILS

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ABSTRACT

Synthetic films materials mostly used to preserve foodstuffs. Recently, attention has turned to biodegradable films because of environmental reasons. The development of new biodegradable packaging material films, for example, chitosan film, is needed to find alternatives to petroleum-based plastics. Chitosan has antimicrobial activities against different groups of microorganisms, such as bacteria, fungus, and yeast. In this project, gelatin is used as the biopolymers in producing the biodegradable film. Gelatin is an attractive alternative to synthetic plastic materials made from non-renewable resources that can be a hazard to the environment. Since gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility, they are expected to form composite films with good properties. The film preparation process consist by hydrating gelatin powder with distilled water for 4 hour and then the samples were heated at 60°C before mixed with chitosan based solution. Then, the solution was poured on a glass plate and left it dried. Lastly, the film was peeled off the glass plate after it completely dried. This work also aimed on the antimicrobial analysis of films against *E. coli* and *B. subtilis*. Method used in this analysis is zone inhibition assay where the inhibition zone diameter is measured and also liquid culture test where the OD reading was measured using spectrophotometer. Based on the study, the clove essential oil has its own antimicrobial activity onto the films. Thus, from the results, it can be concluded that the biodegradable film has smoother surface, higher thermal stability and slightly higher melting point which obtain from SEM, FTIR, TGA and DSC. Gelatin has great potential for being an additive for the composite film and the amount of plasticizer added in the composite film helps to enhance the properties of the film.

ABSTRAK

Filem yang digunakan sebagai bahan pembungkus makanan kebanyakannya diperbuat daripada plastik sintetik. Walaubagaimanapun, disebabkan isu alam sekitar plastik sintetik telah diperbaharui kepada filem biodegradasi. Filem komposit boleh biodegradasi diperbuat daripada chitosan adalah sebagai alternatif. Tambahan pula, chitosan mempunyai antimikroorganisma aktiviti keatas pelbagai kumpulan mikroorganisma sebagai contoh bakteria, kulat dan yis. Di dalam kajian ini, gelatin digunakan sebagai bahan biopolimer di dalam penghasilan filem biodegradasi ini. Gelatin merupakan alternatif yang menarik memandangkan sumber gelatin adalah dari bahan yang tidak membahayakan alam sekitar. Memandangkan gelatin dan chitosan merupakan hidrofilik biopolimer, maka filem komposit ini dijangkakan mempunyai sifat-sifat yang bagus. Penyediaan filem ini merangkumi hidrolisis serbuk gelatin beserta air suling sehingga 4 jam pada suhu sekitar 60°C sebelum ia disebatikan dengan larutan berasaskan chitosan. Larutan yang telah sebati dituang pada kepingan kaca dan dibiarkan pada suhu persekitaran sehingga membentuk filem. Akhir sekali filem dikupas dari kepingan kaca selepas ia telah kering dengan sepenuhnya. Kajian ini juga fokus ke atas antimikroorganisma analisis oleh filem komposit terhadap *E. coli* dan *B. subtilis*. Kaedah yang digunakan di dalam analisis ini adalah kaedah kawasan sisihan oleh bakteria di mana diameter sisihan diukur. Kaedah kedua adalah ujian kultur cecair dimana bacaan OD diukur menggunakan UV-Vis. Berdasarkan keatas kajian tersebut, boleh disimpulkan bahawa filem boleh biodegradasi ini mempunyai permukaan yang licin, kestabilan haba dan takat pelakuran yang tinggi yang diperolehi daripada SEM, FTIR, TGA and DSC. Ini menunjukkan gelatin mempunyai potensi sebagai ejen penambah terhadap filem komposit dan kuantiti ejen plastik juga mampu meningkatkan sifat filem.

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LIST OF ABBREVIATIONS

PVC	Polyvinyl chloride
PEO	Poly ethylene oxide
PEG	Poly ethylene glycol
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared
TGA	Thermo gravimetric analyzer
DSC	Differential scanning calorimeter
Mr	Molecular weight
% v/v	volume percentage for chemical per basis
%w/w	weight percentage for chemical per basis
E. coli	Escherichia Coli
B. subtilis	Bacillus subtilis

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CHAPTER 1

INTRODUCTION

1.1 General Background

Plastic is the general common term for a wide range of synthetic that suitable for the manufacture of industrial products. Plastics are durable and degrade very slowly because the molecular bonds that make plastic so durable make it equally resistant to natural processes of degradation. Since the 1950s, one billion tons of plastic has been discarded and may persist for hundreds or even thousands of years. In some cases, burning plastic can release toxic fumes. Burning the plastic polyvinyl chloride (PVC) may create dioxin. The biggest threat to the conventional plastics industry is most likely to be environmental concerns, including the release of toxic pollutants, greenhouse gas, litter, biodegradable and non-biodegradable landfill impact as a result of the production and disposal of petroleum and petroleum-based plastics (Chen *et al*, 2007).

Nevertheless, for environmental reasons, attention has lately been turned towards biodegradable and edible films to preserve foodstuffs. The materials used to make films are ordinarily waste products from food processing, thereby enhancing processing sustainability. The biodegradable, edible nature of these films meant that they can be employed in both food and agricultural applications (Perez *et al*, 2007). The interest in biodegradable edible films in recent decades can be explained by consumer demand for high quality foods, health factors and environmental concerns over the disposal of non-renewable food packaging materials, and opportunities for creating new market outlets for film e forming ingredients derived from agricultural products (Soares *et al*, 2005). So, biodegradable plastic is better alternatives to petroleum-based plastics. Biodegradable plastics that are break down with exposure to sunlight, water or dampness, bacteria, enzymes or environmental degradation. Starch powder has been mixed with plastic as a filler to allow it to degrade more easily.

In this project the biodegradable film is made from Chitosan and Gelatin. Chitosan is a valuable component of natural packaging films. It is generally obtained from natural chitin after its N-deacetylation by an alkaline treatment. Chitosan is a biodegradable and non-toxic polymer. (Ilona and Barbara, 2006). Chitosan is readily soluble in various acidic solutions such as formic and acetic acids (Chen *et al*, 2007). Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatible in addition to having antimicrobial characteristics. In view of these qualities, chitosan films have been used as packaging material for the quality preservation of variety of food (Dutta *et al*, 2008).

Chitosans are described in terms of the degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjunction with their cationicity and their film-forming properties. Film-making conditions, including solvent pH, ionic strength, type of solvent (acid) used and annealing treatment, are parameters often manipulated to alter the mechanical properties and membrane porosity. Ionic strength or pH can be manipulated in order to reduce inter- and intramolecular electrostatic repulsion between chitosan chains, thus allowing the chains to approach each other and enhance the inter- and intramolecular hydrogen bonding. Chitosan has been extensively used over a wide range of applications, such as a biomaterial in medicine either on its own or as a blend component, a membrane filter for water treatment, a biodegradable, edible coating or film in food packaging a dietary fiber, and a medicine against hypertension because of its scavenging action for chloride ions (Arvanitoyannis *et al*, 1998)

However, in recent years carbohydrates and proteins have been extensively tested to develop biodegradable films having more and more versatile properties. Protein based films offer better mechanical and barrier properties due both to the specific structure of the proteins and the ability of proteins to form stronger intermolecular covalent bonds than carbohydrates (Mateos *et al*, 2007).

In this project, gelatin is used as the biopolymers in producing the biodegradable film. Gelatin is an attractive alternative to synthetic plastic materials made from non-renewable resources that can be a hazard to the environment. Gelatin obtained by partial degradation of collagen has gained more attention as edible films for its abundance and biodegradability. Gelatin has relatively low cost and excellent functional and filmogenic properties. Whereas biopolymers, such as proteins and polysaccharides, provide the supporting matrix, lipids provide a good barrier to water vapor. Since gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility, they are expected to form composite films with good properties (Rivero *et al*, 2008).

Like the collagen, the gelatin chains are macromolecules with a tendency mainly to interchain, rather than intrachain and hydrogen bonding. Gelatin forms a three-dimensional network with zones of intermolecular microcrystalline junctions and the dehydration of this system may produce brittle films. Thus, plasticizers must be added to reduce interchain interactions improving film flexibility (Vanin *et al*, 2005).

Furthermore, in this project there is combination of clove essential oils in producing the biodegradable film. One specific application is to incorporate essential oil into the packaging as antimicrobial agent to prevent the growth of microorganism. The antimicrobial and antioxidant properties of essential oils have been known for a long time, and a number of investigations have been conducted into their antimicrobial activities using various bacteria, viruses and fungi (Dunan *et al*, 2006). Clove oil is a natural preservative and flavoring substances that are not harmful when consumed in food products (Matan *et al*, 2005). Clove oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food.

The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity (Wenqiang, 2006).

1.2 Problem Statement

The most common materials used for packaging are paper, fiberboard, plastic, glass, steel, and aluminum. However, they pose a serious global environmental problem by generating large volumes of non-biodegradable waste. Moreover, in addition to safety and environmental issues, recycling of plastics is complicated for technical and economic reasons. Thus, new biodegradable films made from edible biopolymers from renewable sources could become an important factor in reducing the environmental impact of plastic waste. Proteins, lipids, and polysaccharides are the main biopolymers employed to make edible films and coatings (Guille´n *et al*, 2009).

In this project, chitosan is one of the raw materials. From the previous work, it reported that films made from chitosan lack of water resistance and has poor mechanical properties. Forming biodegradable film from chitosan with other biopolymers is an alternative. So, gelatin is added into chitosan to help the film has good mechanical properties and high tensile strength since gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility).

P.K.Dutta et al, 2008 reported that chitosan has received a significant attention as antimicrobial film-forming agent for food preservation to the researchers due to its biodegradability and antimicrobial activity. Clove essential oil is added in enhancing the antimicrobial analysis of the biodegradable film. The high levels of eugenol contained in clove essential oil inhibit production of an essential enzyme by the bacteria or cause damage to the cell wall of bacteria (Guan *et al*, 2006).

1.3 Objective

The objectives of this study are listed as following;

a. To fabricate composite biodegradable film from chitosan and gelatin.
b. To characterize composite biodegradable film in terms of antimicrobial analysis, morphology and others.

1.4 Scope of Study

The scopes of this study are listed as following;

a. Fabrication of composite biodegradable film from gelatin with chitosan and PEG 400 as additives.
b. The characterization of the composite biodegradable film using various analysis method:-
   i. Fourier Transform Infrared (FTIR) Spectroscopy
   ii. Differential Scanning Calorimetry (DSC)
   iii. Thermogravimetric Analysis (TGA)
   iv. Scanning Electron Microscopy (SEM)
c. The antimicrobial analysis of composite biodegradable film against *E. coli* and *B. subtilis* using these method;
   i. Zone Inhibition Assay
   ii. Liquid Culture Test

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable Film

The use of plastic for packaging has grown extensively in recent years and the use of biodegradable films might be effective in environmental protection. Edible, biodegradable films and coatings, by acting as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavor components, can prevent quality deterioration and increase the shelf life of food products . In addition, edible films or coatings may provide mechanical integrity and improve the handling characteristics of the food. They can be effective carriers of many functional ingredients, such as antimicrobial agents to improve safety and stability of foods, antioxidants to prevent lipid oxidation, and flavorings and pigments to improve quality of foods. Materials that can be used for film making include polysaccharides, proteins, lipids and polyesters or combinations of them (Babak and Oromiehi , 2008).

In recent years, development of biodegradable packaging materials from renewable natural resources has received widespread government support in EU countries and many national or international organizations have been established to facilitate the development in this area. The UK Government-Industry Forum has strongly recommended greater use of nonfood crops, particularly for biodegradable packaging applications. The objectives in the development of biodegradable packaging are two-fold: to utilize renewable and potentially more sustainable sources of raw materials (crops instead of crude oil) and to facilitate integrated waste management approaches so as to reduce landfill. To date, significant technological development has been achieved to produce biodegradable materials for packaging applications with comparable functionalities to those of traditional oil-based plastic packaging (Davis and Song, 2006).

Biodegradable film is the biopolymer films. It is environmentally friendly and could be degraded by microorganisms without further assistance. The films may contain of polysaccharides, protein, lipids and etc. the films easy to degrade due to it’s polymer that built in it is made of organic materials. The biodegradable may consist of one or two organic materials. If the films consist of two organic materials that can be easily biodegrade, it called as biocomposite degradable films. Films based on biopolymers are generally sensitive to the relative humidity of the air since they are normally hygroscopic and have limited mechanical resistance compared with synthetic films. Nevertheless protein-based films display high deformability. A possible solution to improve the mechanical characteristics of protein-based films could be the mixing of these biopolymers with synthetic polymers (Tharanathan, 2003).

Except for plastics related to petrochemical polymers, there are also biodegradable plastics like polylactic acid, but even those could be replaced by polysaccharides due to lower costs and better properties of final composite. Mixing of polysaccharide based materials with plastics means mixing hydrophilic and hydrophobic materials, which requires energy. The goal to replace only a part of the higher-cost plastics with lower-cost polysaccharides is not the best strategy (Rowell *et al.*, 2007).

2.2 Chitosan (Raw Material)

Chitosan, a linear β-1,4-D-glucosamine, is a biocompatible, nontoxic compound mainly obtained by deacetylation of chitin, a natural structural component present for instance in crustaceans such as crabs shells. Several works exist in the literature that demonstrate the inherent biocide properties of this natural carbohydrate polymer against a wide range of microorganisms such as filamentous fungi, yeast and bacteria (Coma *et al*., 2003 and Moller *et al*, 2004).

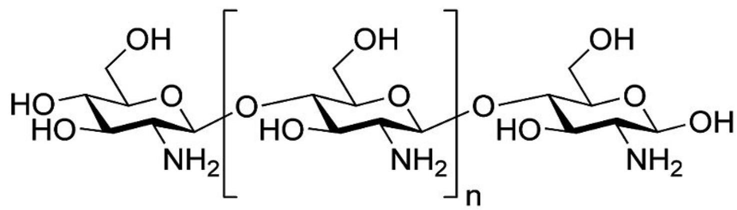

Figure 2.1: Chemical structure of chitosan

Chitosan is a natural polymer, nontoxic, edible, and biodegradable, derived by deacetylation of chitin, which is the second most abundant biopolymer in nature after cellulose. In general, chitin presents in the exoskeleton of arthropods such as insects, crabs, shrimps, lobsters, and certain fungal cell walls. It has a potential as a packaging polymer and, more particularly, as an edible packaging or coating because of its ability to form a film without any use of additives. Moreover, chitosan film has good oxygen and carbon dioxide permeability, which is lower than that of polyethylene film, and good mechanical properties, which are comparable with those of many medium-strength commercial polymers. Furthermore, chitosan has antimicrobial activities against different groups of microorganisms, such as bacteria, fungus, and yeast. Therefore, chitosan has been used in edible coatings or films to extend shelf life of foodstuffs; for example, fruits, meat, and fish and seafood. These studies justify that chitosan might be used as antimicrobial packaging, which is a promising form of active food packaging. Furthermore, the development of new biodegradable packaging material films, for example, chitosan film, is needed to find alternatives to petroleum-based plastics because of environmental concerns (Nugraha *et al*, 2004).

The previous studies have highlighted that several characteristics such as degree of deacetylation, film-forming and storage conditions, molecular weight or the type and concentration of the organic solvent employed, determine the antibacterial effectiveness of the compound. Although, there are over 22,600 publications related to chitin and chitosan since 1907, there is still great controversy regarding the phenomenology and mechanisms of the biocide properties of this natural component. For this reason, there is a critical need to establish more reliable nalytical methods for a proper quality control in the chitosan production, especially regarding molecular weight and degree of deacetylation (No *et al*., 2007).

Chitosan is presently under investigation for a wide range of therapeutic applications, such as burn and wound dressings, sutures, bone fillers, engineered tissue scaffolds, and drug and gene delivery vehicles. Chitosan also has its own advantages such as water binding capacity, fat binding capacity, bioactivity, biodegradability, nontoxicity, biocompatibility and antifungal activity (Yang *et al*, 2004). Chitosan possesses repeating units of 1,4 linked 2-deoxy-2-2-aminoglucose. The amino group NH_2 can be protonated to NH_3 and readily form electrostatic interactions with anionic groups in an acid environment. This property has been applied on edible films (Xu *et al*, 2005).

2.3 Gelatin (Raw Material)

Gelatin is a protein substance derived from collagen, a natural protein present in the tendons, ligaments, and tissues of mammals. It is produced by boiling the connective tissues, bones and skins of animals, usually cows and pigs. Gelatin's ability to form strong, transparent gels and flexible films that are easily digested, soluble in hot water, and capable of forming a positive binding action have made it a valuable commodity in food processing, pharmaceuticals, photography, and paper production.

Gelatin is obtained from collagen by thermal denaturation or physical and chemical degradation (Valeria 2007). Gelatin was one of the first materials employed in formation of biomaterials, and has been subjected in many patents. Gelatin continues to be used in studies on edible films because it is an abundant raw material, produced in the whole world at low cost and has excellent film forming properties. Good revisions on the gelatin structure and its functional properties related to filmogenic abilities have been recently published (Vanin *et al*, 2004).

Gelatin's ability to form thermoreversible gels with a melting point close to body temperature has contributed substantially to an increase in its applications. Gelatin's largest single food use is in gel desserts because of the unique 'melt at mouth temperature, in frozen foods and in dairy products as a protective colloid or stabilizer, i.e. ice crystal inhibitor. Gelatin has also been used in photographic emulsions, playing a multipurpose role such as a protective colloid, ripening agent and binder, in the textile industry as an adhesive and in the pharmaceutical industry for the production of tablets and hard capsules. Food coating and casing applications such as sausage casings and poultry coatings, with or without the presence of antimicrobial compounds, are envisaged as another important and promising issue which has primarily received attention by the meat industry. Occasionally, gelatin has been used in conjunction with other hydrocolloids such as acacia (gum arabic), alginate and pectate esters, soluble and hydroxyl propyl starch (Ioannis *et al*, 1998)

It has gained more attention as edible films for its abundance and biodegradability. Gelatin has relatively low cost and excellent functional and filmogenic properties. Gelatin film itself, as most protein films, does not have ideal water vapor barrier properties. Thus, some chemical treatments can be applied to modify the polymer network through cross-linking of the polymer chains to improve the hydrocolloid film functionality. Food, pharmaceutical and industries are the main users of gelatin, which has several other technical applications. Their most frequent uses in the biomedical field include hard and soft capsules, wound dressings and adsorbent pads for surgical uses, as well as three-dimensional tissue regeneration (Rivero *et al*, 2008).

Like the collagen, the gelatin chains are macromolecules with a tendency mainly to interchain, rather than intrachain and hydrogen bonding. Gelatin forms a three-dimensional network with zones of intermolecular microcrystalline junctions, and the dehydration of this system may produce brittle films. Thus, plasticizers must be added to reduce interchain interactions improving film flexibility (Vanin *et al*, 2005).

2.4 Polyethylene Glycol (PEG)

PEGs are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 300 g/mol to 10,000,000 g/mol. While PEG and PEO with different molecular weights find use in different applications and have different physical properties due to chain length effects, their chemical properties are nearly identical. The two PEG and PEO are chemically synonymous, but historically PEG has tended to refer to shorter polymers with molecular weight Mr < 20,000, polyethylene oxide to high-molecular adducts. PEG undergoes thermo-oxidative and oxidative destruction at the temperature above 310°C, also it is destructed by action of high-speed stirring (Fernandez *et al*, 2006).

The numbers that are often included in the names of PEGs indicate their average molecular weights, e.g. a PEG with n=80 would have an average molecular weight of approximately 3500 daltons and would be labeled PEG 3500. Most PEGs include molecules with a distribution of molecular weights, i.e. they are polydisperse. The size distribution can be characterized statistically by its weight average molecular weight (Mw) and its number average molecular weight (Mn), the ratio of which is called the polydispersity index (Mw/Mn). Mw and Mn can be measured by mass spectroscopy. PEGylation is the act of covalently coupling a PEG structure to another larger molecule, for example, a therapeutic protein (which is then referred to as PEGylated). PEGylated interferon alfa-2a or -2b is a commonly used injectable treatment for Hepatitis C infection (Fernandez *et al.*, 2006).

The composite biofilms of chitosan-polylactic acid incorporated with Polyethylene glycol will make the product more flexible and 'easy to recover' materials. The chitosan-polylactic acid films will have variable thickness depend on their blending mixture and the concentration of polyethylene glycol. The higher the polyethylene glycol, the easier the films of chitosan were removed from polypropylene support and more flexible there were (Se'bastian *et al*, 2006).

The higher concentration of polyethylene glycol in the composite films, the more higher the water vapor transmission rate will be, it is due to progressive film plasticization which is associated with modification of the hydrophilic character of polylactic acid film. The polyethylene glycol, thus decrease the material cohesion by creating intermolecular spaces and increasing water molecule diffusion coefficient or the easier separation of polyethylene glycol with the amorphous phase of polylactic acid which significantly explain the result (Glauser *et al*, 2005). For this research, PEG 400 will be used as the plasticizer.

2.5 Clove Essential Oils

Essential oils also called volatile or ethereal oils are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation, effleurage or extraction but the method of steam distillation is most commonly used for commercial production of EOs. The term ‘essential oil’ is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim; he named the effective component of a drug Quinta essential. An estimated 3000 EOs are known, of which about 300 are commercially important— destined chiefly for the flavors and fragrances market. It has long been recognized that some EOs have antimicrobial properties and these have been reviewed in the past as have the antimicrobial properties of spices but the relatively recent enhancement of interest in ‘green’ consumerism has lead to a renewal of scientific interest in these substances. Besides antibacterial properties, EOs or their components have been shown to exhibit antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties. These characteristics are possibly related to the function of these compounds in plants (Sara, 2004).

Clove (Eugenia caryophyllata Thunb.) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China (Bureau of Drug Administration of China, 1989). Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food.

The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity (Guan 2006).

2.6 Characterization

2.6.1 Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) is a type of electron microscope that can take images of a sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. Electrons from the SEM interact with the atoms of the sample that make up the sample producing signals. These signals contain information about the sample's surface topography, composition and other properties such as electrical conductivity (Kalsom *et al*, 2003).

Figure 2.2: Scanning Electron Microcopy

In order to make it functional the electron microscope must of course have a source of electrons which comprises its illumination system. These illumination electrons are produced by the electron gun. The electron gun consists of three parts, the filament, the shield and the anode. Some of the alternative names for the filament include cathode or emitter (Yakimets *et al*, 2005).

Basically there are two major categories of electron emitters used in SEMs. The first of these represents a class of electron sources that emit electrons as they are heated. These thermionic emitters operate on principal that as certain materials are heated the electrons in the outer orbital become unstable and are more likely to fly free of their atoms.

To create an SEM image, the incident electron beam is scanned in a raster pattern across the sample's surface. The emitted electrons are detected for each position in the scanned area by an electron detector. The intensity of the emitted electron signal is displayed as brightness on a cathode ray tube (CRT). By synchronizing the CRT scan to that of the scan of the incident electron beam, the CRT display represents the morphology of the sample surface area scanned by the beam. Magnification of the CRT image is the ratio of the image display size to the sample area scanned by the electron beam. The SEM column and sample chamber are at a moderate vacuum to allow the electrons to travel freely from the electron beam source to the sample and then to the detectors. This mode provides high-resolution imaging of fine surface morphology. Inelastic electron scattering caused by the interaction between the sample's electrons and the incident electrons results in the emission of low-energy electrons from near the sample's surface. The topography of surface features influences the number of electrons that reach the secondary electron detector from any point on the scanned surface. This local variation in electron intensity creates the image contrast that reveals the surface morphology. The secondary electron image resolution for an ideal sample is about 3.5 nm for a tungsten-filament electron source SEM or 1.5 nm for field emission SEM (Beacom *et al*, 2001).